Antibody Cross-Competition Analysis of the HIV-1 gp120 Envelope Glycoprotein and a Summary of Selected Competition Groups

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Introduction

Here we provide a brief summary of the antibody binding properties of conserved and variable domains of HIV-1 gp120, derived from the paper "Antibody Cross-Competition Analysis of the Human Immunodeficiency Virus Type 1 gp120 Exterior Envelope Glycoprotein," by Moore, J.P. and Sodroski, J., (*Journal of Virology* **70**:1863–1872, 1996), and in prior references cited therein. In that paper, cross-competition studies involving 46 gp120-specific monoclonal antibodies (MAbs) were described, and a detailed competition matrix was assembled. The 46 antibodies can be organized by cross-reactivity patterns into 16 different competition groups. The matrix allows the visualization of the relationships between antibody binding sites both within and between competition groups. Enhancement and inhibition of gp120-binding by antibody pairs are depicted by green and red colors, respectively. From the patterns revealed by the cross-competition matrix, a model was derived that depicts the relationships between the antibody competition groups on the surface of the gp120 molecule in its monomeric form.

The competition matrix is reproduced in Figure 1 with the gracious consent of the American Society for Microbiology.

The competition matrix was derived using recombinant monomeric gp120 from the BH10 clone of HIV-1 LAI. With the exception of MAbs to the V3 and, to a lesser, but still significant extent, the V2 regions, the antibodies selected are fairly broadly reactive among subtype B gp120s, and some show cross-subtype reactivity. All the MAbs used recognize native (non-denatured) gp120, indicating that their epitopes are accessible on the surface of the folded monomeric gp120 molecule. Many of the MAbs used were human, and the rest were rodent (mostly murine). The recombinant CD4-IgG molecule was also used, to represent a bivalent molecule reactive with the CD4-binding site. The sources of reagents are listed in the Moore and Sodroski paper, and can also be found in Part III of this database. Requests for these reagents should be made to those individuals who generated them and kindly donated them for study. There are many similar antibodies to several of these epitopes, for only a representative subset was selected for this analysis. Antibodies used in the study recognize either "linear" epitopes (those mimicked by short peptides), or discontinuous epitopes (not peptide-reactive). The linear epitopes have been described in the previous edition of the database (1995), and other MAbs to them are listed there. The discontinuous epitopes have been added to this edition of the database (1996), and some of their general properties are briefly described here. A non-exhaustive list of references to previous work on these epitope clusters (or MAbs to them) can be found in part III of this database. These papers list other MAbs known (or suspected to be) members of the same competition group. Those who wish their publications and MAbs to be listed in this section in future years are encouraged to contact Dr. Korber with details (email: btk@t10.lanl.gov and fax: (505) 664-3493).

The following is a summary of general features of monoclonal antibodies that form certain of the more interesting competition groups.

Epitopes overlapping the CD4-binding site (CD4BS), represented in the competition matrix by MAbs 15e, 21h, #55, F19, IgG1b12.

Biological activity: These MAbs competitively inhibit CD4 binding to monomeric gp120. Most of these MAbs neutralize T-cell line-adapted strains of HIV-1, probably by inhibition of the virus-CD4 receptor interaction. However, with the exception of IgG1b12, MAbs to these sites lack significant neutralizing activity against primary strains of HIV-1. The precise explanation of this phenomenon remains to be resolved, but it probably includes one, or a combination of, the relative inaccessibility of the MAb epitopes on the oligomeric envelope of primary viruses, and the triggering of the fusion process as a consequence of antibody binding. The potent neutralizing activity of IgG1b12 correlates with its high affinity for the native, oligomeric form of the envelope glycoproteins, compared to other MAbs in this competition group.

Epitope: Many MAbs in this competition group have been identified, mostly of human origin. Most of these MAbs have been derived from HIV-1 infected individuals, since generating these MAbs with other immunization approaches has proven difficult. The conformational epitopes for many of the MAbs have been characterized using a panel of gp120 mutants. Although these studies reveal that the MAbs recognize related epitopes, these epitopes are rarely identical. However, most MAbs in this competition group are unable to bind to gp120 molecules with amino acid substitutions at residues 368 and 370, which are thought to interact with the CD4 molecule.

The CD4-induced epitopes (CD4i), represented in the competition matrix by MAbs 48d and 17b.

Biological activity: MAbs 48d and 17b neutralize T-cell line-adapted strains of HIV-1, but they only have limited activity against the primary viruses against which they have been tested. Recent results suggest that 48d and 17b can inhibit the interactions of gp120 with the CCR5 second receptor, implying that their mechanism of neutralization might be antagonism of stages in the fusion reaction that occur post-CD4 binding. This remains to be confirmed, however.

Epitope: MAbs 48d and 17b (human) are the only members of this competition group yet identified. Their epitopes are constitutively present on monomeric gp120, but are better exposed on the CD4-gp120 complex than on gp120. One possibility is that they overlap with second receptor-binding sites that are exposed on the virus after CD4-binding. Another MAb (CG10, from J.Gershoni, murine, raised to a CD4-gp120 complex) cross-blocks 48d and 17b, but unlike these MAbs, its epitope requires both gp120 and CD4 residues since CG10 binds to neither gp120 nor CD4 alone. The epitopes for 48d and 17b (and CG10) have been studied using the panel of gp120 mutants. The epitopes for 48d and 17b are very similar to one another, but they are not identical. Each epitope is highly complex, for both are sensitive to residues that also influence the CD4-binding site. However, there are also unique features to the CD4i site(s), notably in relation to gp120 changes near the bases of the V1/V2 and V3 loop structures. Indeed, deletion of large segments of these loops exposes the CD4i epitopes, giving rise to the concept that CD4 binding causes a structural change in gp120 that moves the variable loop structures away from the conserved regions of gp120 underneath. The CD4i MAbs are not thought to bind directly to residues within the variable loops, but they are sensitive to certain amino acid substitutions within these loops, presumably reflecting alterations in the efficiency of the conformational changes which occur after CD4 binding that help form the CD4i epitopes. The best estimate of the location of the CD4i epitopes is that they involve relatively conserved structures at the bases of the variable loops.

The second CD4i site, represented in the competition matrix by MAbs A32 and 211/c.

Biological activity: MAbs A32 and 211/c have a very limited ability to neutralize any strains of HIV-1. The probable explanation for this is provided by the observation that their epitopes are not well

exposed on the oligomeric form of the envelope glycoproteins. In the context of monomeric gp120, the epitopes for these MAbs are better exposed after CD4 binding, but the MAbs also bind well to uncomplexed gp120.

Epitope: A32 and 211/c are the human MAbs that are members of this competition group. Two murine MAbs (8F101 and 8F102, from R. Pal), however, have been described that cross-block A32 and 211/c and are sensitive to amino acid substitutions in gp120 that are very similar to those affecting the binding of the human MAbs. The epitopes for A32 and 211/c are present on gp120s from multiple subtypes, implying that they involve conserved regions of gp120. Multiple amino acid substitutions in the C1 and C4 regions of gp120 disrupt these discontinuous epitopes.

The 2G12 site, represented in the competition matrix by MAb 2G12.

Biological Activity: Human MAb 2G12 is one of the most potent neutralizers of primary and T-cell line-adapted strains yet identified. It recognizes an epitope that is well exposed on both monomeric and oligomeric forms of gp120. 2G12 neither interferes with, nor is affected by, CD4 binding, but it is able to inhibit the interaction of gp120 with the CCR5 second receptor, providing a clue as to its possible mechanism of action.

Epitope: 2G12 is the sole known member of its competition group. Its epitope is well conserved across the genetic subtypes, except for subtype E. The binding of 2G12 to gp120 is sensitive to amino acid substitutions in the C2 and C3 regions on either side of the base of the V3 loop, and also to substitutions in the V4 loop. Many of these substitutions affect canonical N-linked glycosylation sites, implying that the 2G12 site might directly or indirectly involve glycans.

The C4/V3 site, represented in the competition matrix by MAbs G3-299 and G3-42.

Biological Activity: These murine MAbs block the binding of monomeric gp120 to CD4. They have a limited ability to neutralize T-cell line-adapted viruses, but have little or no activity against primary strains, probably for reasons similar to those of the CD4BS-related MAbs (see above).

Epitope: One additional member of this competition group is known (13H8 from P. Berman). These MAbs are sensitive to amino acid substitutions in the C4 and V3 regions of gp120 and (especially 13H8) bind to peptides that solely include C4 or V3 residues. The epitope probably spans both regions of the gp120 molecule, which are thought to be closely juxtaposed.

The V2 loop epitopes, represented in the competition matrix by MAbs CRA-3, CRA-4, 684-238, G3-136, G3-4, SC258, BAT-085.

Biological activity: These murine MAbs, and many others that have been identified, have neutralizing ability against T-cell line-adapted strains of HIV-1. However, this is often relatively type-specific and V2 MAbs usually lack neutralizing activity against primary viruses.

Epitope: Several epitope sub-clusters within the V2 loop structure are known, accounting for the complex pattern of interactions between the MAbs in this competition group, and also between them and MAbs in other competition groups. Some of these epitopes are relatively simple (peptide-reactive), including ones in the N-terminal flank of V2 and in the central region of the loop. However, others are discontinuous and are affected by substitutions in several regions of the V2 loop including, but not limited to, the central region.

Antibody Cross-Competition